

a transcriptional repression function domain. However, no other transcribed gene has been found that includes the motif “DLELRL”, and therefore it cannot be said that this motif is a conserved motif. On this account, it has been demanded to find a conserved motif of a transcriptional repression domain like the motif (L/F)DLN(L/F)(X) P, i.e., a conserved motif that is applicable to general plants and conserved in natural transcriptional repressors.

- [0006] [Patent Literature 1]
- [0007] Japanese Patent No. 3829200
- [0008] [Patent Literature 2]
- [0009] Japanese Patent No. 3995211
- [0010] [Patent Literature 3]
- [0011] Japanese Patent Application Publication, Tokukai, No. 2001-269177 A
- [0012] [Patent Literature 4]
- [0013] Japanese Patent Application Publication, Tokukai, No. 2001-269178 A
- [0014] [Patent Literature 5]
- [0015] Japanese Patent Application Publication, Tokukai, No. 2001-292776 A
- [0016] [Patent Literature 6]
- [0017] Japanese Patent Application Publication, Tokukai, No. 2001-292777 A
- [0018] [Patent Literature 7]
- [0019] Japanese Patent Application Publication, Tokukai, No. 2001-269176 A
- [0020] [Patent Literature 8]
- [0021] Japanese Patent Application Publication, Tokukai, No. 2001-269179 A
- [0022] [Non-Patent Literature 1]
- [0023] The Plant Cell, 200113, 1959-1968
- [0024] [Non-Patent Literature 2]
- [0025] Plant Biotechnology J 2006. 4. 325-332
- [0026] [Non-Patent Literature 3]
- [0027] Plant Journal 2003 34:733-739.

SUMMARY OF INVENTION

Technical Problem

[0028] An object of the present invention is to provide a conserved motif sequence serving as a novel transcriptional repression domain available in the CRES-T, which is simple and widely applicable means for repressing transcription of genes, in order to broaden the range to which the CRES-T can be applied and to improve applicability of the CRES-T.

Solution to Problem

[0029] In order to attain this object, the present inventors focused on At2g36080 gene, which is a transcribed gene of *Arabidopsis thaliana*. The present inventors conducted a transient assay on *Arabidopsis thaliana* leaves with use of an effector construct prepared by fusing a GAL4 DNA-binding domain to At2g36080 gene. As a result, the present inventors found that the effector construct has strong transcriptional repression activity, and also found that the region having the transcriptional repression activity was eight peptides “LRLFGVNM”. Next, from all genes registered in the *Arabidopsis thaliana* database, the present inventors found 29 transcriptional regulator genes each including an amino acid sequence analogous to the motif LRLFGVNM. Among these, At3g11580, At2g46870, At1g13260, At1g68840, At4g36990, and At4g11660 were subjected to the same transient assay as above. As a result, all of these genes were

proved to function as transcriptional repressors. Of the transcriptional repressors, six genes had RLFGV as a conserved sequence, whereas At4g36990 had KLFGV as a conserved sequence. At4g36990 was analyzed by using an effector plasmid into which further mutation had been introduced. As a result, five amino acids “K/RLFGV” (first one amino acid is K or R) were determined as a conserved sequence. Meanwhile, the following experiment was carried out: DNA fragments each encoding 15 amino acids, which were a partial fragment of an amino acid sequence encoded by At2g36080 gene and which included RLFGV, were fused to CUC2 gene and AG gene (both of which are transcriptional activators derived from *Arabidopsis thaliana*), respectively, so as to prepare constructs. Then, these constructs were introduced into *Arabidopsis thaliana*. As a result, it was found that fused cotyledons and double flower had been induced therein, as with a case involving use of a publicly-known transcriptional repression domain SRDX (LDLELRLGFA). Thus, the present invention was completed.

[0030] Specifically, the present inventors fused the yeast-derived GAL4 DNA-binding domain to a gene (accession number At2g36080, classified as a transcribed gene in the *Arabidopsis thaliana* database), so as to obtain a chimeric gene serving as an effector construct that expressed under control of the CaMV 35S promoter (35S:GAL4 DBAt2g36080, see A of FIG. 1). Then, the effector construct thus obtained was introduced into *Arabidopsis thaliana* leaves together with a reporter gene including (i) the enhancer region of CaMV 35S and (ii) the GAL4 DNA-binding region (35S-GAL4-TATA-LUC, see A of FIG. 1), and was caused to transiently express therein (transient assay). Consequently, activity of the reporter gene was remarkably repressed, compared with a control (pUC18 or 35S:GAL4 DB, see A of FIG. 1). This suggests that At2g36080 gene functions as a transcriptional repressor (see B of FIG. 1).

[0031] In order to identify the transcriptional repression domain of At2g36080, transient assays were conducted as follows: The coding regions of At2g36080 genes were cut from their respective carboxyl terminuses, and effector constructs including At2g36080 genes having different lengths of coding regions were prepared (see A of FIG. 1). Then, transient assays were conducted with use of these effector constructs, with the result that the amino acid region 178-192 (15-amino acid region) was found to include a region having strong transcriptional repression activity (see B of FIG. 1). Further, only this region was cut out and fused with the GAL4 DNA-binding domain, so that an effector construct was prepared (see A of FIG. 1). The effector construct thus prepared also exhibited strong transcriptional repression activity (see B of FIG. 1). Thus, this region was found to serve as the transcriptional repression domain (repression domain) which imparts transcriptional repression activity to a DNA-binding domain.

[0032] The peptide consisting of these 15 amino acids was subjected to a further detailed analysis. As a result, even only with eight amino acids LRLFGVNM (the amino acids 183-190), the peptide was found to function as the repression domain (see C and D of FIG. 1).

[0033] Next, in all genes registered in the *Arabidopsis thaliana* database, the present inventors searched for genes having a sequence analogous to LRLFGVNM. Consequently, the present inventors found out genes encoding 29 transcriptional regulators, respectively (see Table 1, List of [RK] LFGV). Of these genes, seven genes, At2g36080,